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(54) Particle analyzer.

(57) The invention comprises a particle analyzer for discharging a liquid specimen flow containing particles from a nozzle of a flow cell (14), forming a sheath flow by passing a sheath liquid around the liquid specimen flow, directing a first light beam (e.g. laser beam) at the liquid specimen flow, detecting the first light beam after it has interacted with the particles in the liquid specimen flow, and analyzing the particles on the basis of the detected first light beam, wherein the liquid specimen flow is a flat flow which is thin in the direction of the first light beam and wide in the direction transverse to the first light beam, and the particle analyzer comprises : a one-dimensional image sensor (22) which extends transversely of the particle flow direction for having focused thereon particle image(s) produced by the first light beam and is arranged to be scanned to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor (22) ; and signal processing means (24, 25) for processing the image signals produced by one-dimensional image sensor (22).

The particle analyzer is excellent at calculating morphological information of particles in a liquid specimen such as blood and urine.

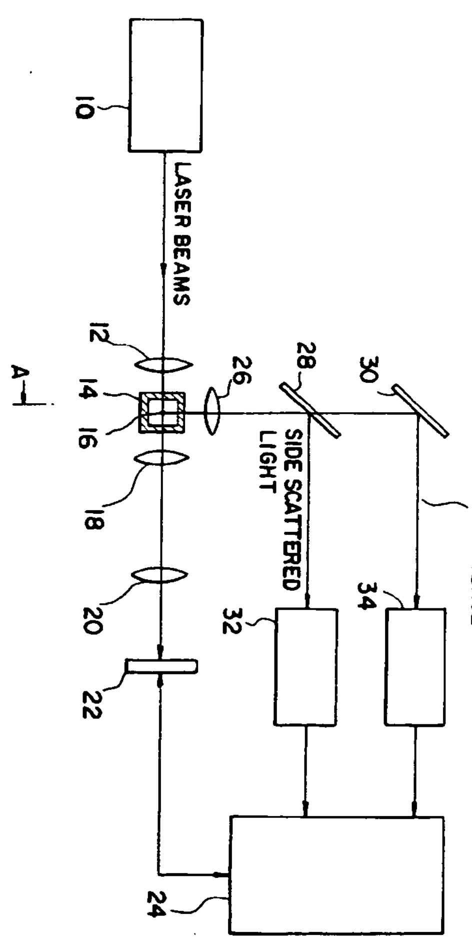

FIG.2

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The present invention relates to a particle analyzer such as a flow cytometer or imaging flow cytometer for analyzing a liquid specimen containing particles such as blood and urine.

Hitherto, as a particle analyzer using a flow cell, an apparatus as shown in Fig. 1 is known. In this apparatus, laser beams from a laser source 100 detect particles flowing in a sheath flow in a flow cell 106. The sheath flow is optically scanned in a direction transverse to the particle flow direction (which is perpendicular to the sheet of paper) by a polarizer (including a lens) 102, and optical signals obtained from the parts of the particles to be analyzed are detected by optical detectors 114, 116, so that more specific morphological information may be obtained for individual particles. Numeral 104 denotes a controller, 108, 110 are objective lenses, 112 is a dichroic mirror, 118, 120 are A/D converters, and 122 is a signal processor. A sheath flow is a flow having the suspension of particles surrounded by a laminar sheath liquid in order to align the particles neatly in a row precisely in the middle of the flow.

In order to scan the laser beams, this apparatus requires an expensive light polarizing element (polarizer 102) and its control device (controller 104) utilizing the acousto-optical effect or electro-optical effect. Besides, the laser beams must usually be made very thin (2 μm or less) and it is necessary to scan the laser beam stably and accurately with respect to the flow of particles.

In the prior art, an apparatus using the so-called slit scan is known, in which laser beams are reduced to about 1 μm and emitted in the flow direction of the particles, and the detection signal waveform of scattered light or fluorescence obtained at this time is analyzed to obtain more specific information about the particles. In this apparatus, specific information separating each particle two-dimensionally is not obtained. That is, it does not possess resolution in the direction transverse to the particle flow direction. Thus, compared with the method shown in Fig. 1 or the apparatus of the invention described below, less information is obtained about the particles and the precision is lower.

On the other hand, Japanese Laid-open Patent Hei. 1-270644 discloses a particle analyzer for scanning light beams in a direction transverse to the particle flow direction, detecting the light transmitted across th particles with a photo detector, and obtaining image information about the particles.

Japanese Laid-open Patent Hei. 2-105041 discloses a particle measuring apparatus for receiving th transmitted light on an array type photo detector disposed at the same position as the detecting part, by improving the apparatus disclosed in the above mentioned Japanese Laid-open Patent Hei. 1-270644.

Japanese Laid-open Patent Sho. 52-113272 discloses an apparatus for scanning a spot of light by using a flying spot tube when passing a biological cell specimen through a flow cell, and obtaining colour information and morphological information (area and shape) of the cells.

Japanese Laid-open Patent Sho. 62-254037 discloses a flow cytometer which has a streak imaging device. The imaging signal is processed only when matched with the predetermined characteristic value, that is, only a particle having a specific characteristic is imaged. The imaging device is a one-dimensional image sensor.

In Japanese Laid-open Patent Hei. 3-123840, a moving object such as iron ore is detected by a one-dimensional image sensor, and the particle size distribution of the object is determined on the basis of the two-dimensional image data obtained by accumulating the one-dimensional image data.

In United States Patent No. 4338024 and Japanese Patent Publication Sho. 57-500995, a flat sample liquid flow is formed, that is, a flat sheath flow is formed, and particle images are taken.

In the apparatus disclosed in Japanese Laid-open Patents Hei. 1-270644, Hei. 2-105041 and Sho. 52-113272, since light beams are scanned, special devices are needed, and stable scanning is difficult.

Japanese Laid-open Patent Hei. 3-123840 discloses an apparatus for measuring the particle size of material such as iron ore added to a blast furnace, and is therefore in a different technical field to the present invention. It makes no mention of real time processing of the accumulated image data or determining parameters such as absorption quantity, area and peripheral length.

In the Japanese Laid-open Patents Sho. 62-254037 and Hei. 3-123840, there is no mention of a flat flow of sample liquid. With a flat flow, the amount of analysis may be increased.

United States Patent No. 4338024 and Japanese Patent Publication Sho. 57-500995 refer to forming a flat sample liquid flow, but the provision of a "one-dimensional image sensor for producing an image signal upon every scan of a flat sample liquid flow, and processing means for processing the image signals" is not mentioned in any one of these seven patent publications.

Besides, as apparatus for detecting and analyzing the features of particles moving in a fluid, the flow cytometer and cell sorter have been hitherto widely known.

In the conventional flow cytometer, the morphological information of particles (area, circumferential length, etc.) could not be obtained. By passing the specimen in a flat sheath flow, emitting strobe light, and processing the image taken by a video camera, the absorption quantity and morphological information of particles may be obtained in real time, but it requires a video camera and complicated image processing device, which means a higher cost. Because the strobe light is operated every frame period (1/30 second) of the video

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camera, particles of low concentration cannot be captured on all image frames at high efficiency. Hence, there are problems in the sample processing ability and repeatability or accuracy of the analysis results.

United States Patent No. 4338024 and Japanese Patent Publication Sho. 57-500995 refer to forming a flat sample liquid flow as stated above, but none of the seven publications mentions anything about a "one-dimensional image sensor for producing an image signal upon every scan of a flat sample liquid flow, and determining absorption quantity and morphological information of the particles by processing the image signals, and determining this information in real time as the particles pass through the detection unit".

According to a first aspect of the present invention, there is provided a particle analyzer for discharging a liquid specimen flow containing particles from a nozzle of a flow cell, forming a sheath flow by passing a sheath liquid around the liquid specimen flow, directing a first light beam at the liquid specimen flow, detecting the first light beam after it has interacted with the particles in the liquid specimen flow, and analyzing the particles on the basis of the detected first light beam, wherein the liquid specimen flow is a flat flow which is thin in the direction of the first light beam and wide in the direction transverse to the first light beam, and the particle analyzer comprises: a one-dimensional image sensor which extends transversely of the particle flow direction for having focused thereon particle image(s) produced by the first light beam and is arranged to be scanned to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor; and signal processing means for processing the image signals produced by the one-dimensional image sensor.

According to a second aspect of the present invention, there is provided a particle analyzer comprising: a light source for directing a light beam at particles in a liquid specimen forming part of a sheath flow; a one-dimensional image sensor which is positionable to extend transversely of the particle flow direction for having focused thereon particle image(s) produced by the light beam and is arranged to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor; and signal processing means for determining characteristics of individual particles by processing the image signals, and analyzing the particles on the basis of the determined characteristics; wherein the signal processing means comprises: background correction processing means for processing each image signal to obtain a corrected signal by calculating the difference between the image signal and a background signal obtained in the absence of any particles in an imaging region of the image sensor; binarizing processing means for processing each corrected signal to obtain a binary signal indicating a particular portion of a particle, the binary signal being produced by comparing the corrected signal with specified threshold data; binary signal processing means for performing logical operation(s) on the binary signal; and arithmetical processing means for calculating the characteristics of particles from the signals produced by one or more of the previous three means.

Embodiments of the invention can, with high precision, obtain morphological information and absorption information of particles in real time, in addition to the optical features of particles (scattered light intensity, fluorescent intensity, etc.) obtained by the conventional apparatus. This is made possible by using a one-dimensional image sensor (line sensor), and analyzing and processing the signals produced by the line sensor.

The invention will now be described by way of non-limiting embodiments with reference to the accompanying drawings, in which:-

Fig. 1 is an explanatory plan view showing an example of a conventional particle analyzer;

Fig. 2 is an explanatory plan view showing a first embodiment of a particle analyzer of the invention;

Fig. 3 is an explanatory diagram showing the beam emitting area and imaging area of the one-dimensional image sensor (line sensor) of Fig. 2;

Fig. 4 is an explanatory diagram showing the particle scanning by the line sensor;

Fig. 5 is an explanatory diagram showing an example of a line sensor detection signal waveform, in which the broken line indicates the threshold level for detecting the nucleus;

Fig. 6 is an explanatory diagram showing an example of a logic waveform after binarizing the waveform shown in Fig. 5, in which the broken line indicates a binarized signal threshold level for detecting the nucleus portion;

Fig. 7 is an explanatory diagram showing two particles crossing the line sensor simultaneously;

Fig. 8 is an explanatory diagram showing the detection signal waveform corresponding to Fig. 7;

Fig. 9 is an explanatory plan view showing a second embodiment of a particle analyzer of the invention;

Fig. 10 is a diagram showing the characteristics of a dichroic mirror;

Fig. 11 is an explanatory diagram showing the laser beam emitting area, the imaging area of the line sensor, and the imaging area of the video camera of Fig. 9;

Fig. 12 is a block diagram showing an example of a signal processor for processing the line sensor signals;

Fig. 13 is an explanatory plan view showing a third embodiment of a particle analyzer of the invention;

Fig. 14 is an explanatory diagram showing the light emitting area and detection area of the one-dimensional image sensor (line sensor) of Fig. 13;

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Fig. 15 is an example of the waveform diagram of background correction processing for the line sensor signals;
Fig. 16 is an example of the waveform diagram for signal processing of the line sensor signals;
Fig. 17 is an explanatory diagram showing the particle scanning by the line sensor;
Fig. 18 is an explanatory diagram showing an example of the waveform of the background corrected signals of successive scanning cycles;
Fig. 19 is an explanatory diagram showing an example of the logic waveform produced by binarizing the signal shown in Fig. 18 by using a threshold level Thl;
Fig. 20 is an explanatory diagram showing an example of the waveform produced by an AND operation on the signal of Fig. 19;
Fig. 21 is an explanatory diagram showing an example of the logic waveform produced by binarizing the signal shown in Fig. 18 by using a threshold level Th2;
Fig. 22 is an explanatory diagram showing an example of the waveform produced by an AND operation on the signal of Fig. 21;
Fig. 23 is an explanatory diagram showing an example of the waveform produced by an exclusive-or operation on the signal shown in Fig. 19;
Fig. 24 is an explanatory diagram showing an example of the waveform produced by an exclusive-or operation on the signal shown in Fig. 20;
Fig. 25 is a block diagram showing an example of a calculating circuit for obtaining parameters in real time; and
Fig. 26 is a block diagram showing an example of a signal processing device.

Embodiment 1

Fig. 2 is a plan view of the particle analyzer of Embodiment 1. This apparatus is based on the conventional flow cytometer, and is combined with a detection system of a one-dimensional image sensor 22 (hereinafter called line sensor 22), and its signal processing means 24 (hereinafter called signal processing device 24).

A stained (dyed) specimen is led into a flow cell 14 composed of a transparent body of glass, plastic or the like, and a sheath liquid is supplied in such a manner as to cover or surround the specimen, and a flat sheath flow is formed. The laser beam from the laser source 10 is reduced by a cylindrical lens 12 so as to be thin in the particle flow direction (the direction perpendicular to the sheet in Fig. 2), and broad in the direction at right angle to the particle flow direction (the vertical direction in Fig. 2), and crosses a specimen flow 16. When the particle to be analyzed crosses this laser beam, the light reaching the line sensor 22 is weakened. That is, the image of the light passing through the particle is focused on the surface of the line sensor 22 through objective lens 18 and projection lens 20, and signals corresponding to the exposure quantity for individual picture elements of the line sensor 22 are sequentially generated.

The time required for generating signals for all picture elements is determined by the number of picture elements and the clock frequency for shifting signals of all elements of the line sensor, and in the case of, for example, 256 picture elements and clock frequency of 12 MHz, it takes about 20 μsec. This time is the tim required for scanning the transmission images for one line, that is, the scan cycle.

Fig. 3 is a magnified view of the specimen liquid flow portion as seen from the direction of arrow A in Fig. 2. As shown in Fig. 3, in relation to the laser beam emission area 58, the line sensor imaging area 56 is located almost in the center of the emission area 58. Numeral 60 denotes a particle.

The state of particle scanning by the line sensor 22 is shown in Fig. 4, and the detected signal waveform of the line sensor 22 in Fig. 5. By processing this detected signal, the morphological information and absorption information of the particle are obtained. The morphological information includes parameters such as area of cell, circumferential length, area of nucleus (rate), circumferential length, width of cell, complicated degree inside cell (complicated quantity/area), and roundness. Besides, the absorption information includes parameters such as absorption quantity, absorbance (absorbtive degree) (absorption quantity/area).

In addition to such parameters of morphological information and absorption information, by using the signals detected by the photo detectors 34, 32, other information such as fluorescent intensity or side scattered light intensity, the particle may be analyzed at a higher precision by using this information. Numeral 26 is an objective lens, and 28, 30 are dichroic mirrors.

In the apparatus of Fig. 2, however, the sheath flow velocity is limited to around 100 mm/sec. by the scanning cycle and image resolution in the particle flow direction. This flow velocity is many times slower than in the conventional flow cytometer, and it is feared that the number of cells analyzed per unit time may be smaller.

To avoid this problem is far as possible, it may be considered to flatten the sample liquid in the flow cell 14 to increase the volume of sample flowing in the detecting part in unit time. For example, as compared with

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a conventional round sheath of 15 μm in diameter (columnar sheath), a flat sheath of 15 x 150 μm is used, and sample volume per unit time is increased about 13 times for the same flow velocity. However, if the concentration of particles to be analyzed is high, the possibility of simultaneous passing of plural particles in the detecting part increases. Thus it is necessary to extend the width of the laser beam in the detecting part, which results in lower emission intensity, and therefore the following counter-measures may be considered in a system requiring to capture low intensity fluorescence.

(1) To raise the laser power.

(2) To reduce the laser emission width in the direction of particle flow.

(3) To use a high sensitivity photomultiplier.

Concerning the increase of simultaneous passing rate by flattening the specimen flow, if the simultaneous passing rate in the case of round sheath is 0.5%, that of flat sheath is 5%, for example. When two or more particles pass simultaneously side by side as shown in Fig. 7, in the slender detecting area (imaging area) 56, the signal value of the scatter intensity or fluorescence intensity obtained in the detecting system of the conventional flow cytometer does not correspond to one particle. Besides, it is difficult to judge from the signal waveform whether the particles have passed simultaneously or not. On the other hand, the detected signal of the line sensor 22 is as shown in Fig. 8, and from this detected signal waveform it is easy to judge whether there is simultaneous passing or not.

On the basis of the result of judgement, it may be controlled to ignore the optical information obtained in the case of simultaneous passing. By flattening the specimen flow, the simultaneous passing rate increases to 5% and, if such particles are ignored, the substantial increasing effect of the quantity of the sample to be analyzed is still great. In addition, by flattening the flow of liquid specimen, it is also effective to align the flat cell orientation hydrodynamically.

Concerning the flattening of liquid specimen, a further consideration is required in the sectional shape of the flow cell. The conventional flow cell for flat sheath flow is flat as disclosed in U.S. Patent No. 4338024 or Japanese Patent Publication Sho. 57-500995, but in this shape it is impossible to obtain the side fluorescence or side scattered light detected by the conventional flow cytometer. Accordingly it gives rise to the necessity of the flow cell for flat sheath that is not too flat. That is in the conventional flow cell of large aspect ratio (the vertical size is much larger than the lateral size, for example, scores of times, and the degree of flatness is large), the side light signal cannot be detected, and therefore the flow cell of small aspect ratio (the vertical size is somewhat larger than the lateral size, for example, one to several times, and the degree of flatness is small) is needed. As the apparatus for forming such flat sheath flow, the present applicant has already invented the apparatus for gradually narrowing only the width of one direction of the passage of a flow cell inlet, opening the discharge port of the sample nozzle flatly, and matching the shorter direction of the discharge port with the decreasing direction of the inlet passage, and the apparatus for disposing sheath liquid dividing means for dividing symmetrically the sheath liquid into two flows in contact with the sample nozzle, and positioning the discharge port of the sample nozzle in the sheath liquid converging region downstream of the sheath liquid dividing means (see Japanese Patent Applications Hei. 3-210053 and Hei. 3-210054).

Instead of forming a round sheath flow by using the flow cell 14 shown in Fig. 2, by forming a flat sheath flow by using the flat sheath flow cell 15 as shown in Fig. 9, the capacity of analysis per unit time may b increased.

Embodiment 2

Fig 9 shows the particle analyzer of Embodiment 2. This apparatus is based on the apparatus of Embodiment 1, but includes a second light source 36 such as strobe light source (hereinafter called strobe light source 36) for obtaining further particle images, an imaging system composed of second capturing means 38 such as video camera (hereinafter called video camera 38), and an image processing device 40. Numeral 37 is a power source for strobe. That is, on the basis of the optical information and morphological information obtained in the apparatus of embodiment 1, each particle is analyzed in real time, and when the particle is judged to be the particle to be observed closely, the strobe light source 36 is triggered, and the flowing particle is illuminated instantly, and the particle image is captured by the video camera 38.

The light from the strobe light source 36 is transformed into parallel light by a collimator lens 47, and only the light excluding the light of the spectrum below the near ultraviolet rays is reflected by the mirror 44, and passes to the imaging area of the video camera 38 through the lens 46. The light transmitted through the particle is reflected by the mirror 48 through the objective lens 18, and is focused on the CCD plane of the video camera 38.

When strobe light is emitted at this time, in order that the side fluorescence or side scattered light of this light may not be detected, it is desired to use a photomultiplier with gate function as the photo detectors 52,

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50. That is, for the period of 1 or 2 μsec. of illumination of strobe light, the gate is applied so that the photomultiplier may not function, or it is exempted from signal proc ssing during the strobe illumination period. Numeral 25 is a signal processing device, and 47 is a projection lens.

The wavelength of the laser beam emitted from the laser light source 10 is, for example, 441.6 nm in the case of helium-cadmium laser, and by using the dichroic mirrors 44, 48 which pass the light of this wavelength (441.6 nm), but do not pass light of more than this wavelength, the laser beam and the light for video camera imaging are separated. Since this wavelength is at the end of the visible light region, the color image of a particle may be captured with a sufficiently satisfactory color quality (tone of color). A characteristic of the dichroic mirror is shown in Fig. 10.

The relation between the imaging area of the line sensor and the imaging area of the video camera in the embodiment is shown in Fig. 11. As shown in the diagram, the imaging area 56 of the line sensor and the imaging area 58 of the laser beam are located nearly in the center of the imaging area 54 of the video camera, and the imaging system of the video camera 38 may be added to the same optical axis system as the optical axis system in Embodiment 1, so that the optical system may be composed in a compact form. This is possible because the detected signal processing of line sensor is done in real time, the parameters of morphological information and absorption quantity are obtained within 100 μsec. after the particle 60 crosses the imaging area 56 of the line sensor, and the type of particle can be judged on the basis of these parameters, fluorescence intensity, and/or side scattered light intensity, and in the case of sheath flow velocity of 100 mm/sec. the particle moves only 10 μm in 100 μsec. Meanwhile, if the detecting part of the flow cytometer and the imaging area of the video camera are apart from each other, a delicate control is required on the stability of the sheath flow velocity and the timing of image capture.

Given below is the outline about the processing of the detected signal by the line sensor 22 in embodiment 1 and Embodiment 2. An example of detected signal waveform obtained by the line sensor is shown in Fig. 5. For processing such signal, an example of block diagram of signal processing circuit for obtaining the morphological information and absorption information of particle is shown in Fig. 12.

The signal from the line sensor 22 is amplified by the amplifier 62, and is A/D converted by the A/D converter 64 at a sampling clock at the same frequency as the shift clock of the line sensor, and the data is corrected and processed in the background correction circuit 66. In the background correction circuit 66, the background data of one scanning cycle obtained when the particle is not passing through the detecting part is preliminarily stored in the memory, and the difference between the background data and A/D converted data obtained during measurement is obtained and fed out, all by real time processing. It is the object of this processing to correct the shading of laser beam emission and fluctuations of sensitivity of each picture element of the line sensor.

The corrected data is compared with data of a certain proper reference level in order to cut out the portion corresponding to the transmitted light image of the particle, and is binarized in the binarizing unit 68. Furthermore, in order to cut out only the portion of the nucleus stained (dyed) at high density, the data is also compared with the data of larger level than the reference level, and is binarized. An example of binarized signal waveform is shown in Fig. 6.

The binarized signal is pretreated in the binary signal processing unit 70 in order to remove small debris and divide the region of binary signal corresponding to individual particles. The particle region division processing in this case is to cut out the region (timing) of the binary signal corresponding to one particle appearing in plural continuous line data, and this processing is necessary for creating the timing control signal for calculating the morphological information or absorption information of individual particles in real time.

The arithmetic unit 74 for obtainning the absorption quantity, complicatedness and morphological information is controlled by the control signal from this particle region division processing and arithmetic control circuit 72, and the parameters for individual particles may be calculated in real time.

From the binary signal processed signals, simultaneous passing of particles is judged in the simultaneous passing judging unit 76, and it may be controlled so as to ignore the parameters obtained in this case. Numeral 78 is a differentiator, 80 is an absorption quantity arithmetic unit, and 82 is a complicatedness arithmetic unit. The complicatedness is calculated by summing up the differences of A/D converted adjacent data within a range corresponding to one particle (complicated quantity), and dividing it by the area. As the complicated quantity, meanwhile, the squares of the differences of adjacent data may be summed up in a range corresponding to one particle.

The embodiment described in Fig. 2 through Fig. 12 is thus composed, and brings about the following effects.

(1) In addition to the optical features of the particles obtained in the conventional flow cytometer (scattered light intensity, fluorescence intensity, etc.), the morphological information of particles may be obtained in real time, and a particle analysis of higher precision is realized. Besides, since the specimen is formed in

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a flat sheath flow and measured, the possibility that two or more particles pass side by side to cross the monitoring area of the line sensor increases, but by processing the detected signal of the line sensor, this state can be judged, and therefore it is possible to control so as to ignore the fluorescence signal and scattered light signal obtained during the simultaneous passing of particles (the sample width may be increased, and the number of particles that are analyzed may be increased).

(2) Since the sample flow including the particles is flat, when the strobe light source and video camera are added, only the particles that must be inspected particularly in detail among many types of particles are sorted with high precision and image-captured. Besides, by high speed signal processing of the detected signal by the line sensor, the morphological information of particles can be obtained in real time, and therefore the capturing area of the video camera may contain the detection area as the flow cytometer and the detection area of the line sensor, so that the optical system may be formed in a compact design. It is not necessary to consider the deviation of the timing caused by division of the imaging area as in the prior art, and it is possible to capture particle image securely in spite of changes in flow velocity.

(3) The expensive polarizer element and its driver circuit required in the conventional system are not needed.

(4) When the apparatus of the invention is applied in the conventional cell sorter, by adding the particle morphological information and absorption quantity or absorbance, in addition to the optical information obtained by prior art, as the information for the judgement of sorting the particles may be separated with higher precision.

Embodiment 3

Fig. 13 shows a plan view of the particle analyzer of Embodiment 3. The specimen including the particles to be analyzed is led into a sheath flow cell 216 and a flat liquid specimen flow 217 is formed. That is, it flows in a flow cell 216 as a flat specimen flow thin in the direction of optical axis, and broad in the direction at right angle to the optical axis. The specimen flows in a direction vertical to the sheet of paper in Fig. 13. The flow cell 216 is formed of a transparent body such as glass or plastic.

The light from the light source 210 is transformed into parallel light by a collimator lens 212, and is reduced into a slender form by a cylindrical lens 214, and is emitted to the specimen flow region of the flow cell 216.

As the flow cell, a flat shape as disclosed in the U. S. Patent No. 4338024 or the Japanese Patent Publication Sho. 57-500995 may be used, but they cannot detect the side light signal because the aspect ratio of flow cell is too large (the vertical size is too large for the lateral size, for example, scores of times, and the flatness is large). Accordingly, a flow cell of small aspect ratio is required (the vertical size is slightly larger than the lateral size for example, one to several times, and the flatness is small). As the apparatus for forming such flat sheath flow, the present applicant has already invented apparatus for gradually narrowing only th width of one direction of the passage for flow cell inlet, opening the discharge port of the sample nozzle flatly, and matching the shorter direction of the discharge port with the decreasing direction of the inlet passage, and apparatus for disposing sheath liquid dividing means for dividing symmetrically the sheath liquid into two flows in contact with the sample nozzle, and positioning the discharge port of the sample nozzle in the sheath liquid converging region downstream of the sheath liquid dividing means (see Japanese Patent Applications Hei.3-210053 and Hei. 3-210054).

Therefore, as the flow cell 216 shown in Fig. 13, it is preferred to use the flow cell with the small aspect ratio.

Fig. 14 is a magnified view of the portion of the liquid specimen flow 228 as seen from the direction of arrow B in Fig. 13. The light 232 reduced into a slender elliptical form is emitted to the line sensor detection area 234. The light from the line sensor detection area 234 is focused on the light receiving plane of the one-dimensional image sensor 222 (hereinafter called the line sensor 222) by the objective lens 218 and projection lens 220. In Fig. 14, W1 denotes the width of liquid specimen flow, and W2 is the width of the detection area 234 of the line sensor.

The detected signal by the line sensor 222 while the particle 230 is not crossing the detection area 234 of the line sensor is signal Sa as shown in Fig. 15 (a). The signal waveform at this time should be ideally a flat straight line, but actually the signal waveform is undulated due to sensitivity fluctuations of picture elements of the line sensor, or shading of light illumination in the longitudinal direction of the detection area of line sensor.

In a certain scan cycle i, while the particle 230 is crossing the detection area 234 of the line sensor, the light is blocked (weakened) by the particle, and therefore the image signal Sb(i) as shown in Fig. 15 (b) is obtained. By subtracting the signal Sb(i) from the signal Sa, as shown in Fig. 15 (c), the signal Sc(i) being rid of the fluctuations of picture elements of the line sensor and effects of shading is obtained. This is called the background correction processing. Furthermore, by comparing the signal Sc(i) with the reference level Th,

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the binary signal Sd(i) indicating the presence of particle is obtained as shown in Fig. 15 (d). In actual processing, this processing is done digitally. That is, just before start of measurement, by converting signal Sa from analog to digital as shown in Fig. 16 (a) to (d), the waveform data Saj of time series is obtained, and the data Saj is stored in the memory, and similarly the difference between the data Saj and the time series waveform data Sbj(i) of the signal Sb(i) obtained after start of measurement is calculated digitally in real time to determine Scj(i). This background corrected data Scj(i) is compared with proper reference data (threshold level) Th, in order to extract only the portion of the particle, thereby obtaining binary data Sdj(i).

Explained next is how to obtain the information such as absorption information and morphological information in real time from the detected signals by the line sensor.

Fig. 17 shows a scanning state for a certain particle 230 by the line sensor. Numeral 231 is a nucleus. The figures in parentheses in Fig. 17 denote the scan cycle i. At this time, the signal obtained in each scan cycle i is subjected to the above background correction, and the data S1(i) shown in Fig. 18 is obtained. The value summing up the area of the shaded portion in Fig. 18 is equivalent to the absorption quantity of the particle. The absorption quantity may be divided by the particle area to obtain the absorbance.

Meanwhile, the threshold levels Th1, Th2 in Fig. 18 are levels for extracting the whole particle and the nucleus of particle, respectively. By summing up the area (indicated by shaded zone) of the portion where the data S1(i) is over the threshold level Th2, the absorption quantity of the nucleus portion may be obtained. When the nucleus is stained (dyed)by Feulgen's method, the absorption quantity and the DNA quantity are in satisfactory correlation, so that useful information is obtained.

Fig. 19 shows the logic signal S2(i) when the signal shown in Fig. 18 is binarized by threshold level Th1 for particle extraction. The area of the particle is obtained by using this signal, but when the periods of high levels of binary signals are summed up directly, the result is a little larger than the area of the actual particle. Accordingly, as shown in Fig. 20, the AND operated signal S3(i) between the binary signal S2(i-1) obtained in scan cycle i-1 and the binary signal S2(i) obtained in the present scan cycle i is obtained sequentially, that is, S2(i-1) x S2(i), and it is known that the value is closer to the actual value of particle area by summing up the high level periods of these binary signals S3(i). Actually, the sum value is multiplied by the distance data L of the particle moving in one scan cycle to obtain the area.

If the particle to be analyzed has a nucleus inside, such as a leukocyte, the threshold level Th2 for extracting only that nucleus portion is set separately, and by processing the binarized signal in comparison with the level, the area of the nucleus portion may be obtained similarly (see Figs. 21, 22).

The method of calculating the approximate value of the particle width and circumferential length is explained below. The exclusive-or operated signal (EXOR) S6(i) between the binary signal S2(i-1) obtained in scan cycle i-1 and the binary signal S2(i) obtained in the present scan cycle i is successively obtained, as shown in Fig. 23. The value obtained by summing up the high level periods of this signal is almost equivalent to twice the width in the direction vertical to the moving direction of particle (X-direction). Accordingly, the half of th value may be regarded as the particle width in the X-direction. Meanwhile, S6(i) is determined in the following Formula,

$$S6(i) = S2(i-1) \oplus S2(i)$$
$$\leqq S2(i-1) * \overline{S2(i)} + \overline{S2(i-1)} * S2(i)$$

Besides, from this Fig. 23, the approximate value of the circumferential length of the particle is obtained by summing up the square root of the addition of the square of each EXOR signal pulse and the square of the particle moving extent in one scanning cycle. The accuracy is higher when the number of scanning times for one particle is greater or the moving distance of particle in one scan cycle is shorter. Fig. 24 shows an example of ANEX waveform of exclusive-or operated signals S7(i) between adjacent scan cycles of AND signal shown in Fig. 20. A method of calculating the width and circumferential length of particle by using those signal S7(i) is similar.

From thus obtained value, the roundness and area rate of nucleus can be also calculated. As the roundness, generally, the squared value of the circumferential length divided by the area is widely used. This value is the smallest in the case of a circle, and is larger as the shape of the particle is slender. For identical shapes, the value is the same regardless of the size.

As other parameters obtained by processing the detected signal by the line sensor, it may be also considered to express the value obtained by differentiating the detected signal, adding the value and dividing by the area as the complicatedness inside the particle.

As an example of arithmetic circuit for calculating the above parameters actually in real time, an example

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of an arithmetic circuit for calculating the area, circumferential length, roundness and nucleus area rate is shown in Fig. 25.

When the AND signal S3(i) expressing the portion of the particle (Fig. 20) is high, the counter CNT1 counts the number of sampling clock pulses, and when the width of all AND signals S3(i) corresponding to one particle is counted, the value is entered in the lookup table LUT1. In this lookup table LUT1, the value corresponding to the particle moving extent L predetermined by the dip switch DIPSW is also entered, and when the two sets of data are input, the multiplied value is output from the LUT1 within 150 nsec. The coefficient changeover signal entered in the lookup table LUT1 is changed over depending on the magnitude (size) of the particle to be analyzed. This is intended to suppress the data of wide dynamic range by the size of particle to the data in the number of bits easy to handle by the data analyzer.

Likewise, the width of all AND signals S5(i) corresponding to the nucleus portion of one particle is counted by the counter CTN2, and the value is entered in the lookup table LUT2. In the LUT2, the data from the counter CNT1 is also entered, and the rate of the area of the nucleus portion corresponding to the area of the entire particle is output from the LUT2.

On the other hand, when the EXOR signal S6(i) or S7(i) becomes high, the counter CTN3 begins to count, and when the signal changed from high to low, the result of counting is transferred to the lookup table LUT3, and the counter CNT3 is cleared. In the LUT3, the value corresponding to the particle moving distance in one scanning cycle is also entered from the dip switch DIPSW, and the data of the square root of the sum of the squares of these two pieces of input data is instantly output from the LUT3. The output data is added, by accumulator ACC, for every EXOR signal pulse. The value obtained by summing up all EXOR signal pulses of EXOR signal pulses corresponding to one particle is produced as approximate data of circumferential length.

Thus obtained circumferential length and area data are entered in the LUT4 and the data of (circumferential length) ^{2}/area is produced instantly from the LUT4 as the roundness.

The lookup table (LUT) herein refers to the result of calculation which is written in memory beforehand as the numerical table, and it is the general means where real time arithmetic processing is required. The tim from the input of the data till output of the data depends on the access time of the memory.

Fig. 26 is a block diagram of an embodiment of the entire processing circuit (signal processing means) 224 for processing the detected signals by the line sensor 222 and calculating the parameters. The detected signal by the camera 236 with the line sensor 222 is amplified, and is fed into the A/D converter 240 through a filter 238 for removing noise and high frequency components due to the shift clock of the line sensor or the like. The A/D conversion is effected in synchronism with the shift clock, and the data is transferred to a background correction processing circuit 242. This circuit 242 possesses a line memory for holding the data of one line (one scanning cycle) while the particle does not cross the line sensor detection area before start of measurement, and the difference from the data of each scan cycle after start of measurement is calculated. Thus corrected data is binarized in the binarizing processing circuit 244 possessing two sets of reference data (threshold levels) Th1, Th2. The binarized data is sent to binary signal processing circuit 246 to be pretreated for calculating the area and circumferential length, that is, logical operation such as AND, exclusive-or operation between two lines (scanning cycles) of binary signal, and pretreatment for dividing (regional dividing) of the signal of the portion corresponding to one particle. The processed data is transferred to the regional division processing part 248 and arithmetic unit operation control circuit 250.

The regional division processing in this case is the processing for determining whether each binary signal pulse indicating the part of particle belongs to which particle, and it appears in the continued plural lines (scanning cycles). It is intended to deliver the range of binary signal corresponding to one particle as one arithmetic control signal. If two or more particles, close to each other, pass through the detection area of the line sensor, it is controlled to assign which set of arithmetic unit to which particle when plural sets of arithmetic units are installed so as not to overlook the particles.

In the arithmetic unit operation control unit 250, using the regional division signal and signal from the binary signal processing unit, signals are created to control the operation of the arithmetic unit of each parameter. That is, when the binary signal, AND signal, EXOR signal or ANEX signal is changed from low to high, it is controlled to start or continue the operation of each arithmetic unit, and when the signal is changed from high to low, a signal is produced for controlling to end or interrupt the operation of each arithmetic unit. Besides if two or more particles pass the detection area close to each other, when plural sets of arithmetic units are provided so as not to overlook the particles, it is controlled to change over the sets of the arithmetic unit to be operated for every signal corresponding to the particles. Besides, the control signal for obtaining the absorption quantity is also generated from the arithmetic unit operation control unit 250.

The absorption quantity arithmetic unit 252 is intended to sum up the data after background correction processing. The degree of complicatedness is obtained by calculating the difference of the adjacent data by the differentiator 245, summing up the difference data by the complicatedness arithmetic unit 254, and dividing

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the sum by the area. The morphological information arithmetic unit 256 is a circuit as shown in Fig. 25, for example. A counter circuit 258 is intended to count the particles detected by the line sensor.

In this way, the parameters obtained in real time by the signal processing device 224 every time the particle passes through the detection area of the line sensor are transferred to the data analyzer 226 (see Fig. 13), and the particles are analyzed and classified.

To obtain the particle width and circumferential length, it is explained above to get EXOR signal between binary signals S2(i) and S2(i-1), but it is also possible to use AND signal S3(i) between binary signals S2(i) and S2(i-1), and obtained from ANEX signal by EXOR-operation between the AND signals S3(i) and S3(i-1).

(1) When obtained by using EXOR signal--to have EXOR between S2(i) and S2(i-1):

The X width is obtained accurately. The circumferential length tends to be longer than real length by about twice the moving extent in one scanning cycle.

(2) When obtained by using ANEX signal--to have EXOR between S3(i) and S3(i-1):

The X width tends to be smaller than the real width. The circumferential length tends to be slightly smaller than the real length.

It is not possible to decide which method is better. Anyway, the accuracy of approximate value is higher when the moving distance of cell in one scanning cycle is shorter.

As explained in Fig. 13 through Fig. 26, the embodiment is thus composed, and hence brings about the folloiwng effects.

(1) The approximate values of the absorption quantity and morphological information of moving particles may be obtained in real time without using expensive video camera or image processing system.
(2) By adding the detection system by line sensor and signal processing device of the invention to the conventional flow cytometer or cell sorter, novel feature parameters for individual cells are obtained, and it is possible to classify the particles with higher precision.
(3) By adding the detection system by line sensor and signal processing device of the invention to th conventional imaging flow cytometer, only the particles of interest are sorted out, and the desired particles may be efficiently captured by the video camera and edited.
(4) If the particle concentration of sample liquid is high, by installing plural sets of arithmetic units, up to about 10,000 particles per second may be analyzed in real time.
(5) By flattening the liquid specimen flow, the orientation of flat cells may be aligned hydrodynamically.

Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art without departing from the invention.

Claims

1. A particle analyzer for discharging a liquid specimen flow containing particles from a nozzle of a flow cell, forming a sheath flow by passing a sheath liquid around the liquid specimen flow, directing a first light beam at the liquid specimen flow, detecting the first light beam after it has interacted with the particles in the liquid specimen flow, and analyzing the particles on the basis of the detected first light beam, wherein the liquid specimen flow is a flat flow which is thin in the direction of the first light beam and wide in the direction transverse to the first light beam, and the particle analyzer comprises:

a one-dimensional image sensor (22) which extends transversely of the particle flow direction for having focused thereon particle image(s) produced by the first light beam and is arranged to be scanned to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor (22); and

signal processing means (24, 25) for processing the image signals produced by the one-dimensional image sensor (22).

2. A particle analyzer according to claim 1, further comprising a second light source (36) for producing a second light beam, and second imaging means (38) for capturing two-dimensional still images of particles in the liquid specimen flow, wherein:

a one-dimensional imaging region (56) of the one dimensional image sensor (22) is located in a two-dimensional imaging region (54) of the second imaging means (38); and

the signal processing means (25) is arranged to detect the presence of a particle on the basis of the image signals from the one-dimensional image sensor (22) and then to operate the second light source (36) to capture a two-dimensional still image of that particle.

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3. A particle analyzer according to claim 1 or 2, wherein the signal processing means (24, 25) is arranged to determine whether the image signals from the one-dimensional image sensor (22) indicate the presence of a plurality of particles and, in response to a positive determination, to instruct that data concerning scattered light and/or fluorescent light from the particles is ignored.

4. A particle analyzer according to any one of claims 1 to 3, wherein the signal processing means (24, 25) is arranged to determine particle morphological information and/or absorption information on the basis of the image signals from the one-dimensional image sensor (22).

5. A particle analyzer according to claim 4, wherein the morphological information is one or more of the cell area, cell circumferential length, nucleus area, cell width, cell complexity, cell roundness, and nucleus area fraction.

6. A particle analyzer according to claim 4 or 5, wherein the absorption information is one or more of the absorption quantity and the degree of absorption.

7. A particle analyzer comprising:

a light source (210) for directing a light beam at particles in a liquid specimen (217) forming part of a sheath flow;

a one-dimensional image sensor (222) which is positionable to extend transversely of the particle flow direction for having focused thereon particle image(s) produced by the light beam and is arranged to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor (222); and

signal processing means (224) for determining characteristics of individual particles by processing the image signals, and analyzing the particles on the basis of the determined characteristics;

wherein the signal processing means comprises:

background correction processing means (242) for processing each image signal (Sb(i)) to obtain a corrected signal (Sc(i)) by calculating the difference between the image signal (Sb(i)) and a background signal (Sa) obtained in the absence of any particles in an imaging region (234) of the image sensor (222);

binarizing processing means (244) for processing each corrected signal (Sc(i)) to obtain a binary signal (Sd(i)) indicating a particular portion of a particle, the binary signal (Sd(i)) being produced by comparing the corrected signal (Sc(i)) with specified threshold data (Th);

binary signal processing means (246) for performing logical operation(s) on the binary signal (Sd(i)); and

arithmetical processing means (245, 248, 250, 252, 254, 256) for calculating the characteristics of particles from the signals produced by one or more of the previous three means (242, 244, 246).

8. A particle analyzer according to claim 7, wherein the arithmetical processing means comprises:

first processing means (248, 250) for, in respect of each scan cycle, obtaining an AND signal (S3(i)) by performing a logical AND operation on the binary signal (S2(i)) of the scan cycle (i) and the binary signal (S2(i-1) of the preceding scan cycle (i-1); and

means (256) for multiplying the sum of the widths of the AND signals corresponding to one particl by the distance (L) moved by the particle in one scan period, thereby to obtain the area of the particle.

9. A particle analyzer according to claim 7 or 8, wherein the arithmetical processing means comprises:

second processing means (248, 250) for, in respect of each scan cycle, obtaining an EXOR signal (S6(i)) by performing an exclusive-or operation on the binary signal (S2(i)) of the scan cycle (i) and the binary signal (S2(i-1)) of the preceding scan cycle (i-1); and

means (256) for, in respect of each particle, calculating the width of each EXOR signal, calculating the square root of the sum of the square of each EXOR signal width and the square of the distance (L) moved by the particle in one scan period, and calculating the cumulative sum of the square roots, thereby to obtain the circumferential length of the particle.

10. A particle analyzer according to any one of claims 7 to 9, wherein the arithmetical processing means comprises:

second processing means (248, 250) for, in respect of each scan cycle, obtaining an EXOR signal (S6(i)) by performing an exclusive-or operation on the binary signal (S2(i)) of the scan cycle (i) and the binary signal (S2(i-1)) of the preceding scan cycle (i-1); and

means (256) for dividing by 2 the sum of the widths of the EXOR signals corresponding to one

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particle, thereby to obtain the width of the particle in the direction perpendicular to the direction of movement of the particle.

11. A particle analyzer according to any one of claims 7 to 10, wherein the arithmetical processing means comprises:

means (252) for obtaining the cumulative sum of the corrected signals (S1(i)) corresponding to one particle, thereby to obtain the absorption quantity of the particle.

12. A particle analyzer according to any one of claims 7 to 11, wherein the arithmetical processing means comprises:

third processing means (248, 250) for, in respect of each scan cycle, obtaining an AND signal (S3(i)) by performing a logical AND operation on the binary signal (S2(i)) of the scan cycle (i) and the binary signal (S2(i-1)) of the preceding scan cycle (i-1), and obtaining an ANEX signal (S7(i)) by performing an exclusive-or operation on the AND signal (S3(i)) of the scan cycle (i) and the AND signal (S3(i-1)) of the preceding scan cycle (i-1); and

means (256) for, in respect of each particle, calculating the width of each ANEX signal, calculating the square root of the sum of the square of each ANEX signal width and the square of the distance (L) moved by the particle in one scan period, and calculating the cumulative sum the square roots, thereby to obtain the circumferential length of the particle.

13. A particle analyzer according to any one of claims 7 to 12, wherein the arithmetical processing means comprises:

third processing means (248, 250) for, in respect of each scan cycle, obtaining an AND signal (S3(i)) by performing a logical AND operation on the binary signal (S2(i)) of the scan cycle (i) and the binary signal (S2(i-1)) of the preceding scan cycle (i-1), and obtaining an ANEX signal (S7(i)) by performing an exclusive-or operation on the AND signal (S3(i)) of the scan cycle (i) and the AND signal (S3(i-1)) of the preceding scan cycle (i-1); and

means (256) for dividing by 2 the sum of the widths of the ANEX signals corresponding to one particle, thereby to obtain the width of the particle in the direction perpendicular to the direction of movement of the particle.

14. A particle analyzer according to claim 8, further comprising a second such signal processing means (224) using different specified threshold data (Th2) in order to determine characteristics of the nuclei of particles instead of the characteristics of the entire particles, the second signal processing means (224) including means for dividing the nucleus area of a particular particle by the particle area of that particle thereby to obtain the nucleus area fraction.

15. A particle analyzer according to claim 14, wherein the second signal processing means (224) includes means (252) for obtaining the cumulative sum of the corrected signals (S1(i)) corresponding to the nucleus of one particle, thereby to obtain the absorption quantity of that nucleus.

16. A particle analyzer according to claim 8, wherein the arithmetical processing means further comprises:

differentiating means (245) for obtaining the difference between successive corrected signals (S1(i)); and

means (254) for calculating the sum of the differences corresponding to one particle, or the sum of the square of the differences, and dividing the sum by the area of the particle, thereby to obtain the complexity per unit area.

17. A particle analyzer according to claim 9 or 12, wherein the arithmetical processing means further comprises means for squaring the circumferential length and dividing by the area, thereby to obtain the roundness of the particle.

18. A particle analyzer according to any one of claims 7 to 17, wherein each signal processing means (224) comprises a plurality of arithmetical processing means so that characteristics may be calculated simultaneously if a plurality of particles simultaneously cross the imaging region (234) of the one-dimensional imag sensor (222).

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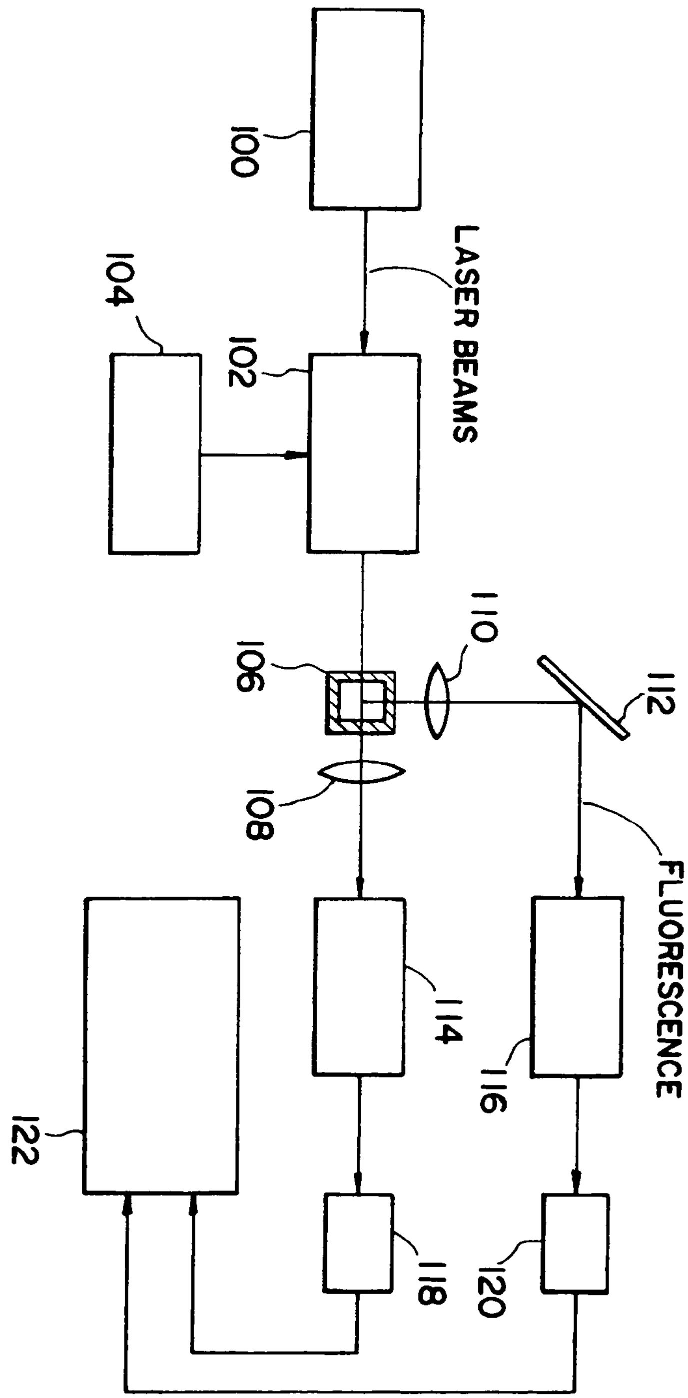

FIG.1 PRIOR ART

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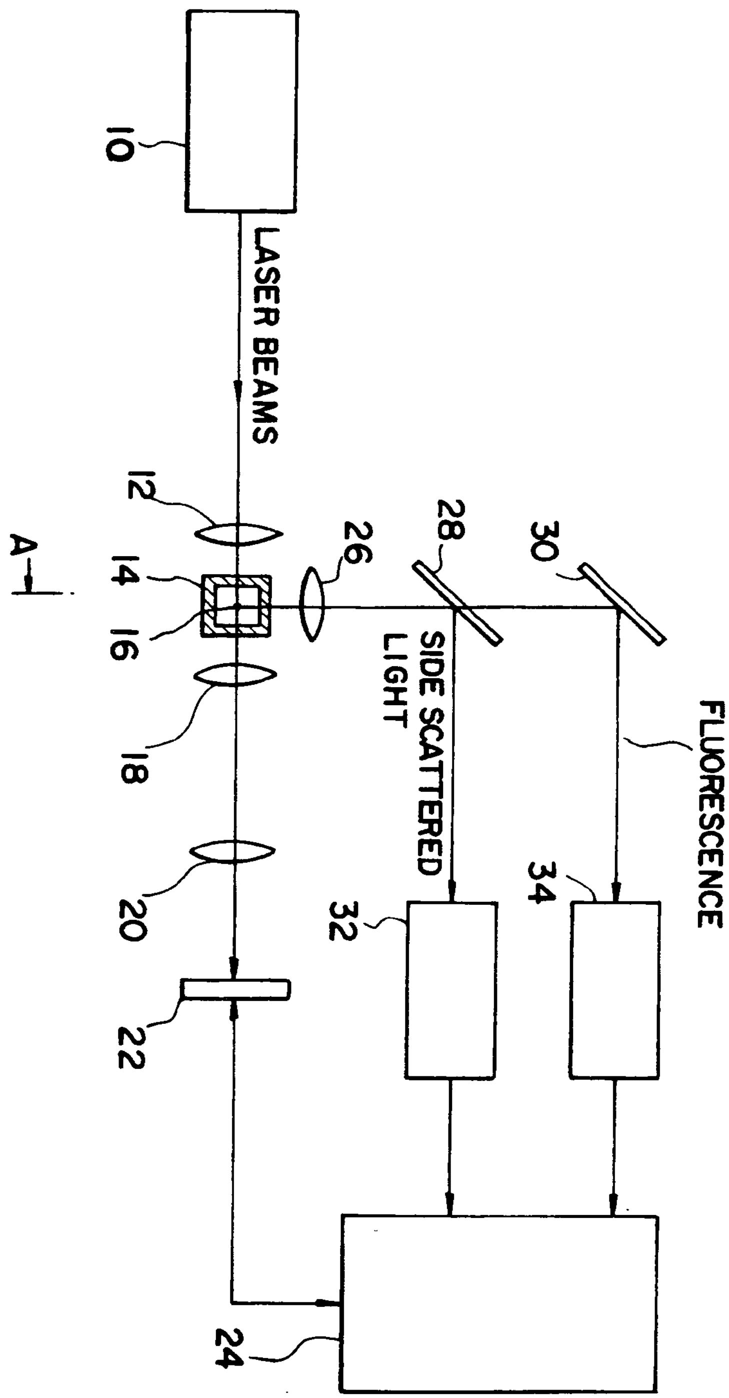

FIG.2

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FIG. 3

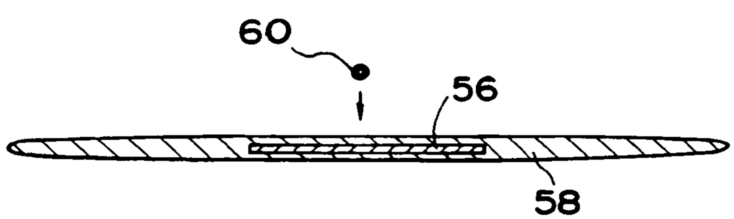

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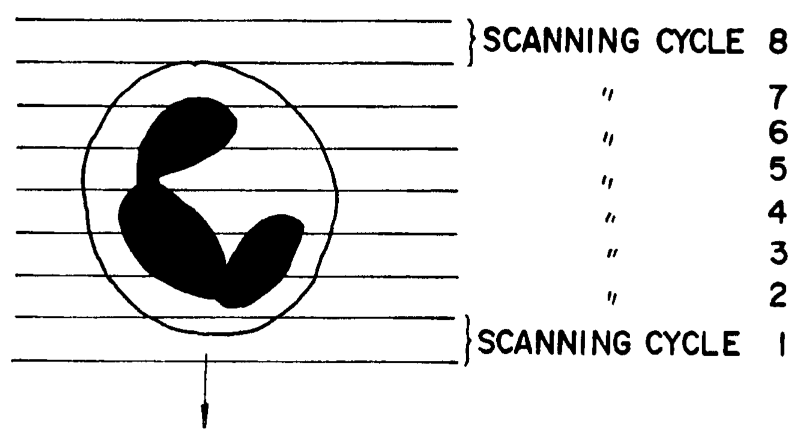
FIG.4



SHEATH LIQUID FLOW VELOCITY : 50~100mm/sec

RESOLUTION OF FLOW DIRECTION : 1~2 μm

RESOLUTION OF HORISONTAL DIRECTION : 0.5~1 μm (ON USING LINE SENSOR OF 256 PICTURE ELEMENTS)

SCANNING CYCLE : 20~30 μsec

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FIG.5

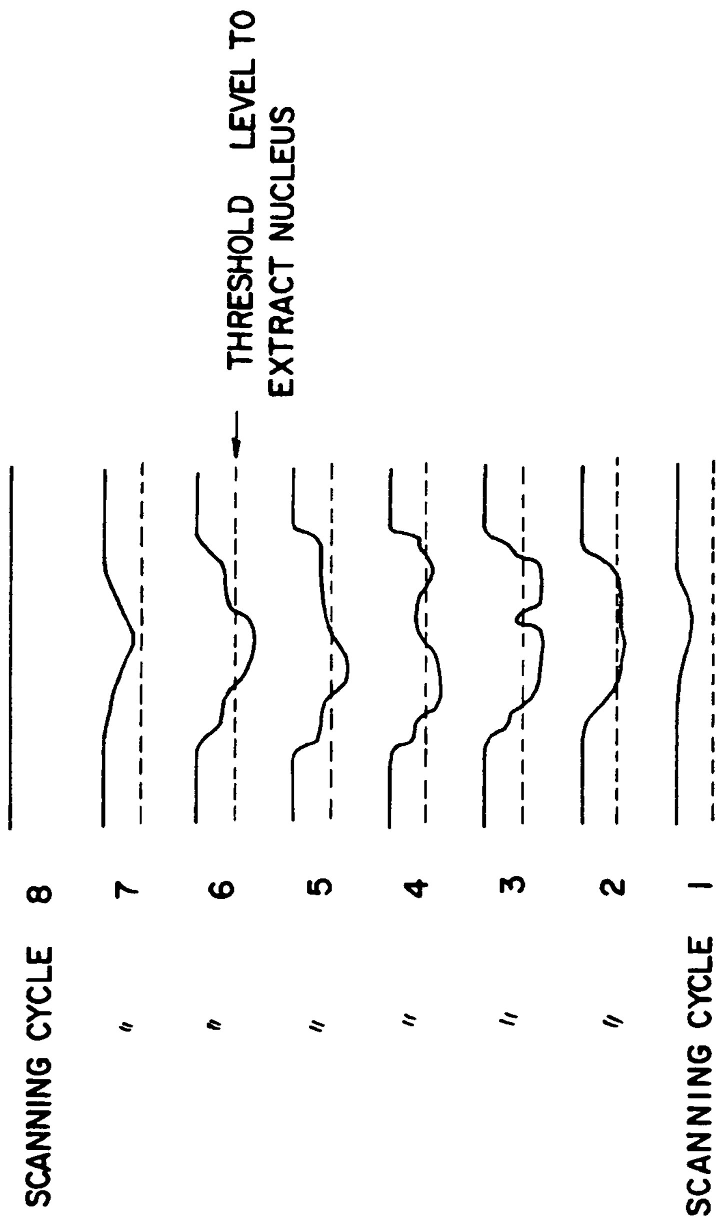

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FIG.6

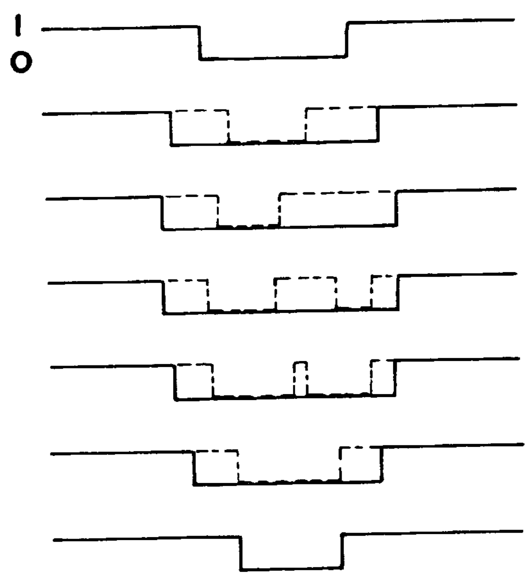

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FIG. 7

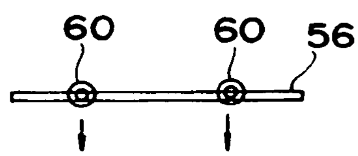

FIG. 8

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FIG.9

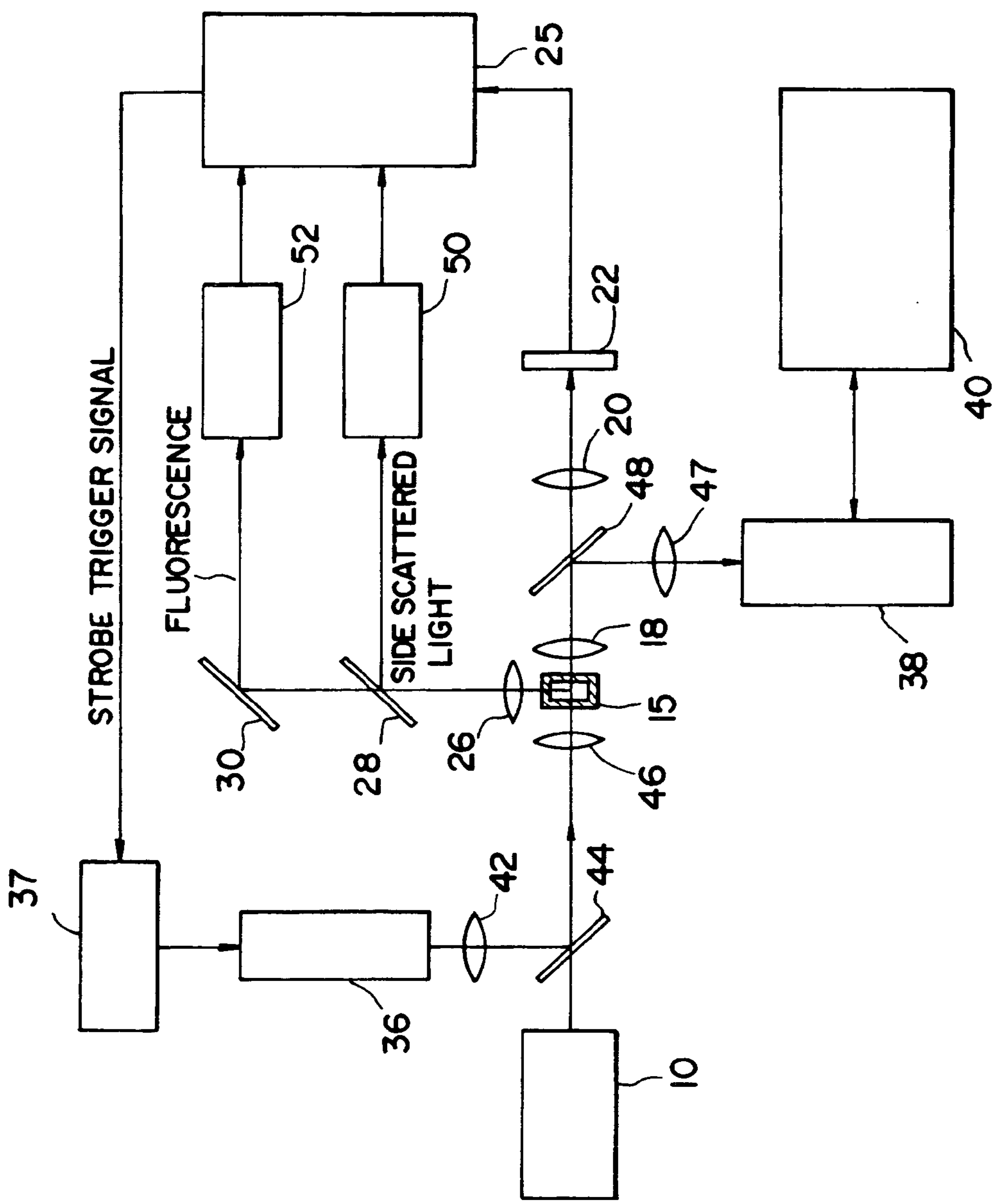

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FIG.10

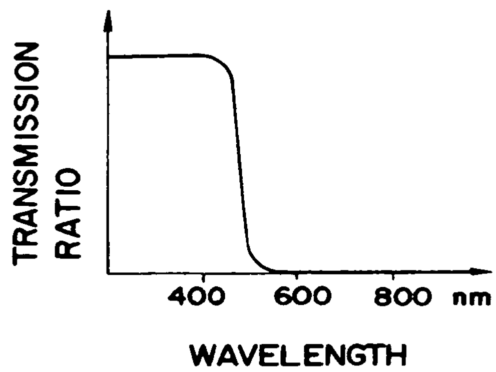

FIG.11

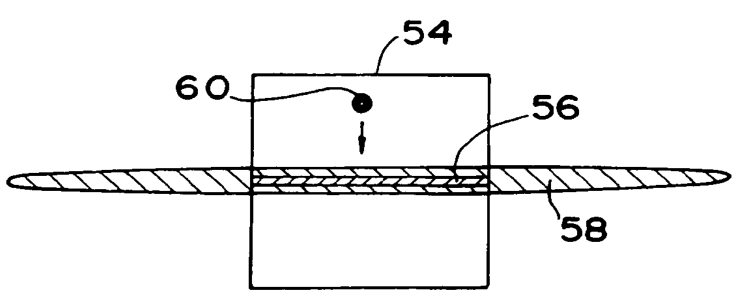

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FIG.12

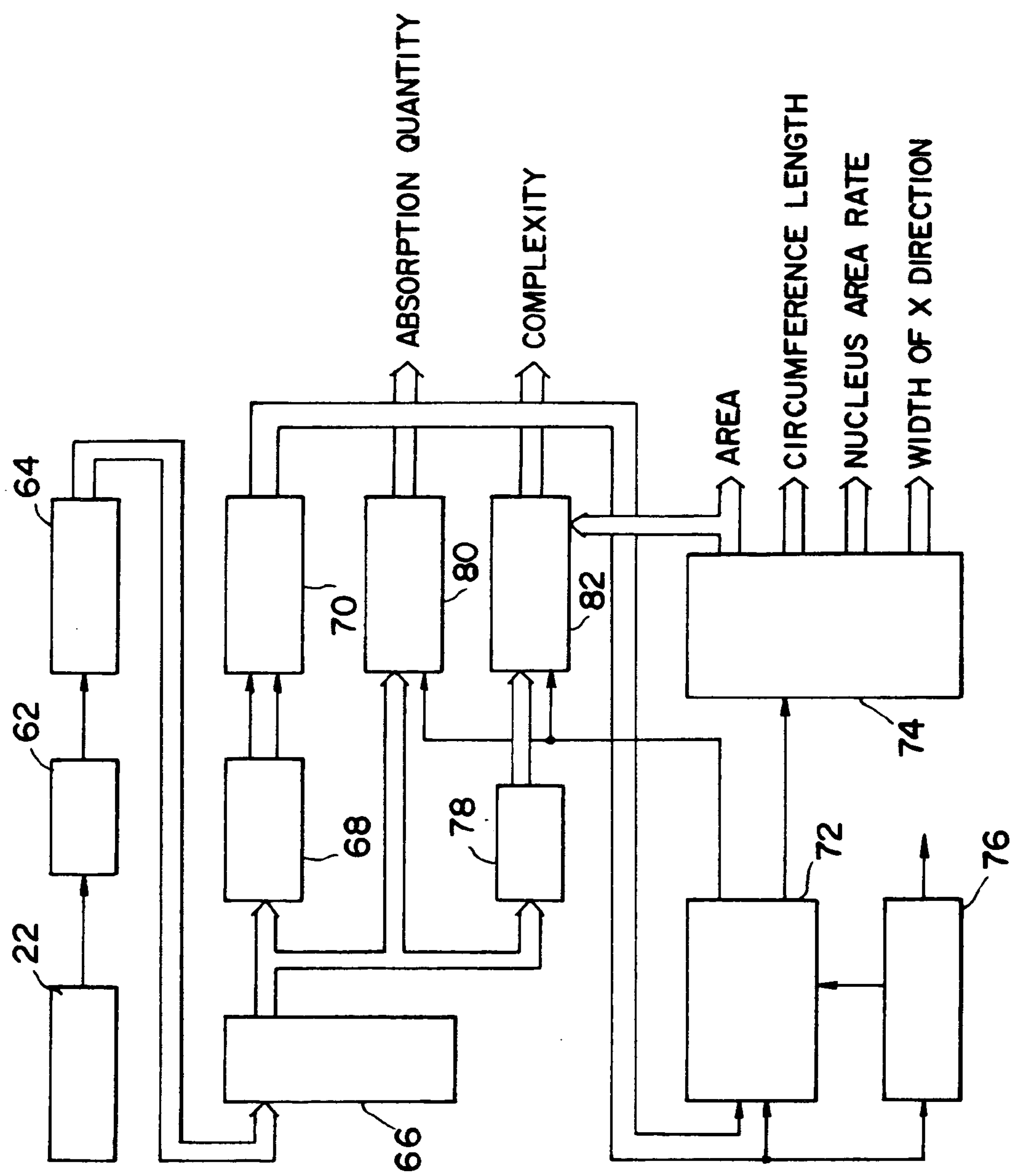

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FIG.13

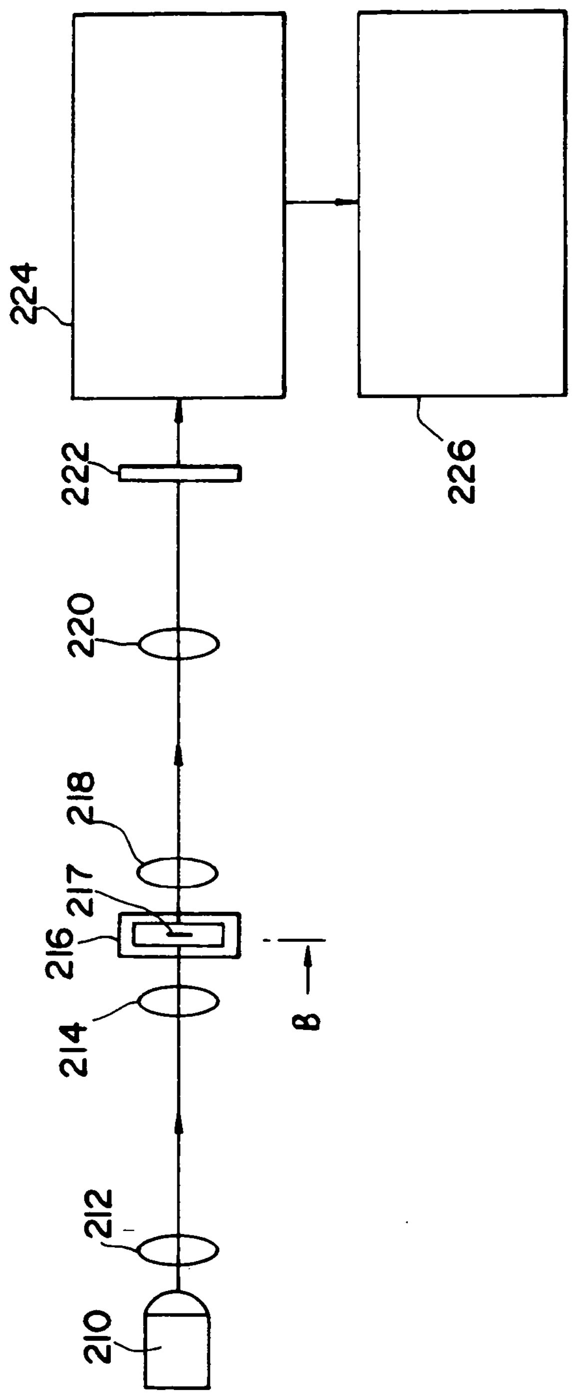

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FIG.14

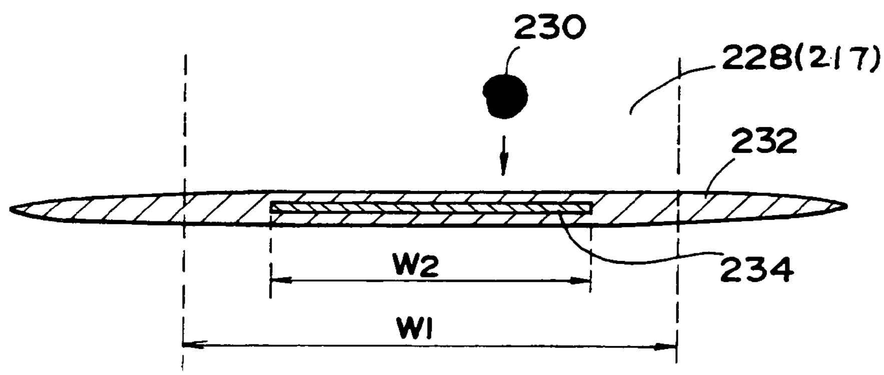

FIG.15

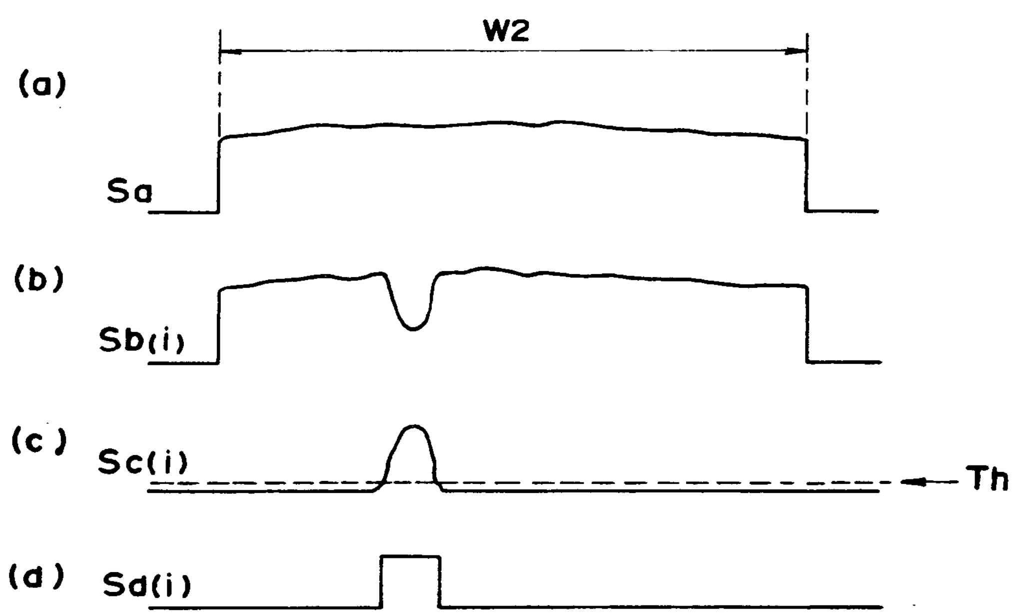

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FIG.16

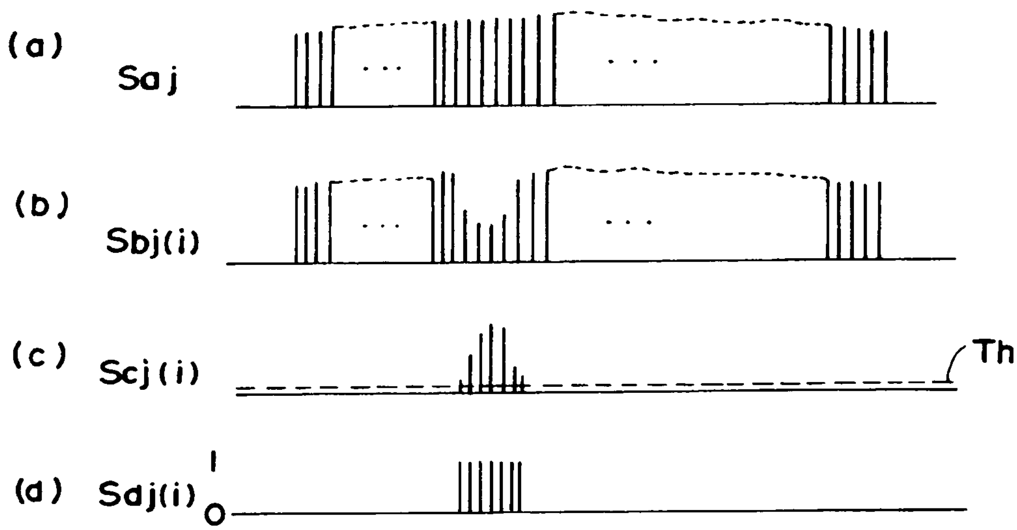

FIG.17

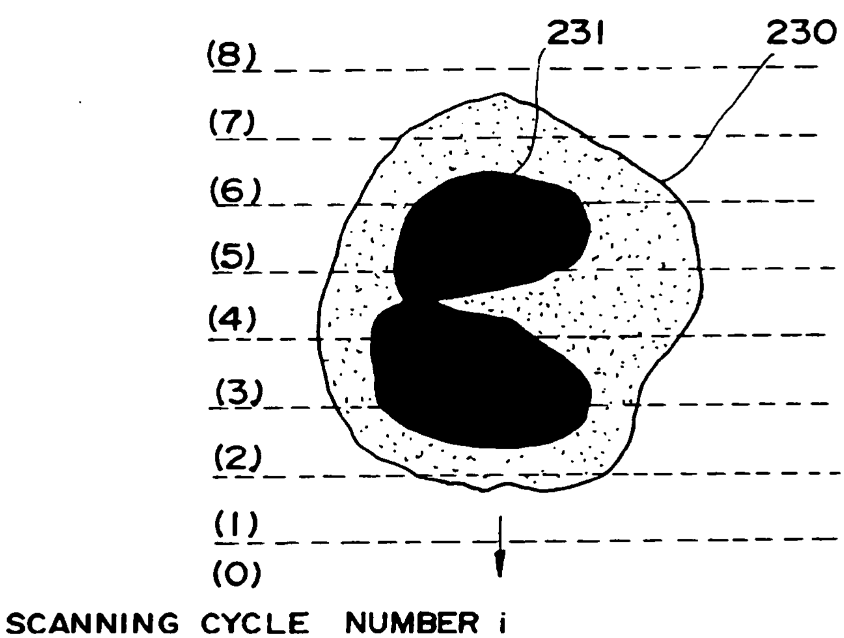

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FIG.18

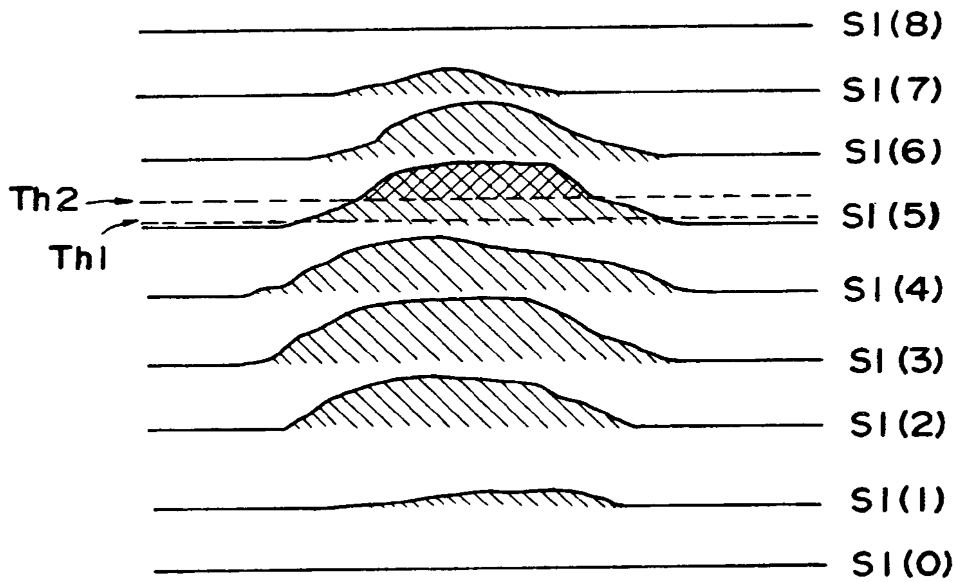

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FIG.19

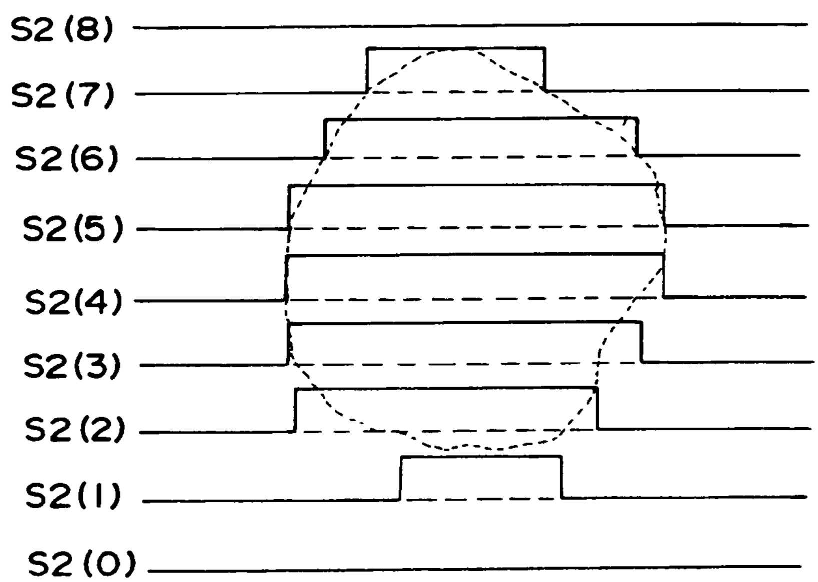

FIG.20

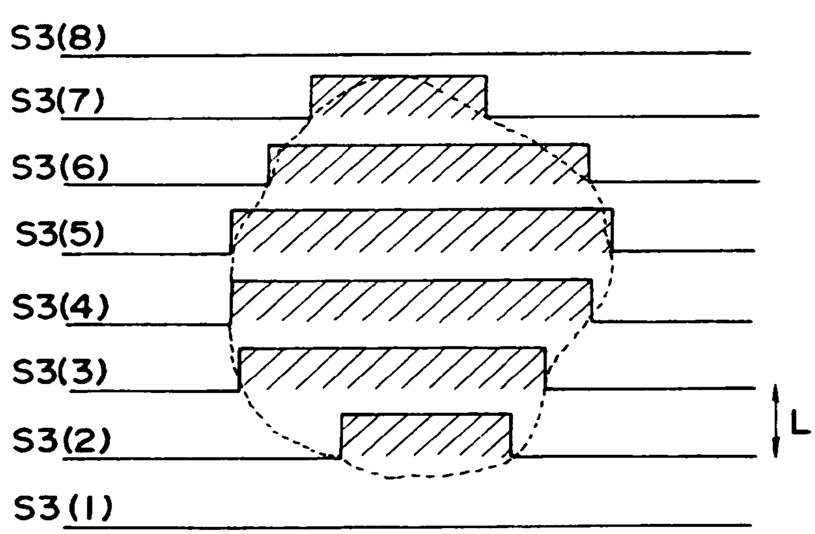

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FIG.21

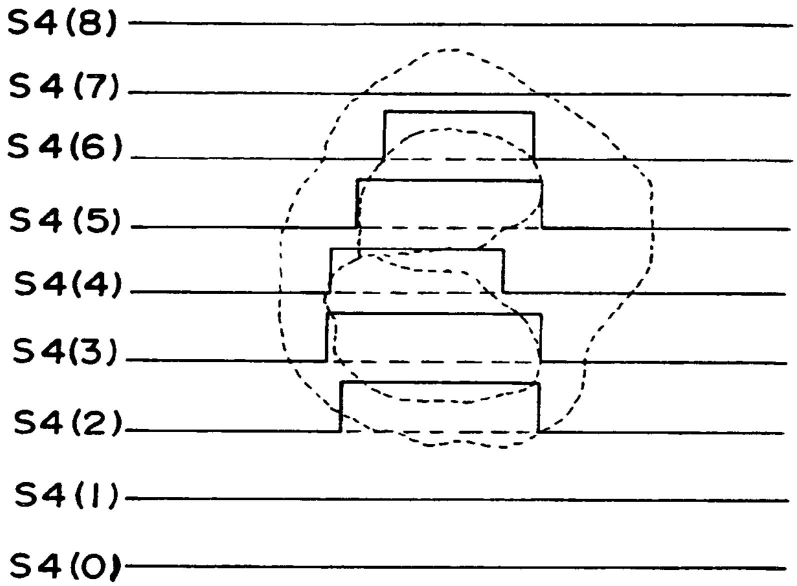

FIG.22

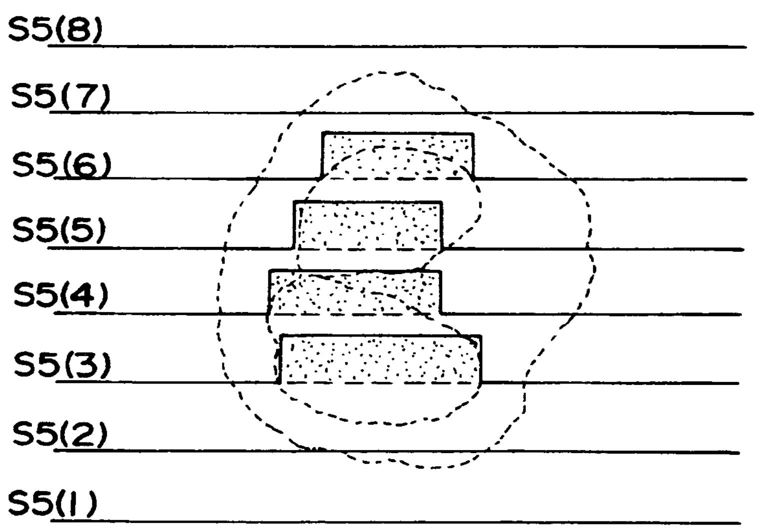

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FIG.23

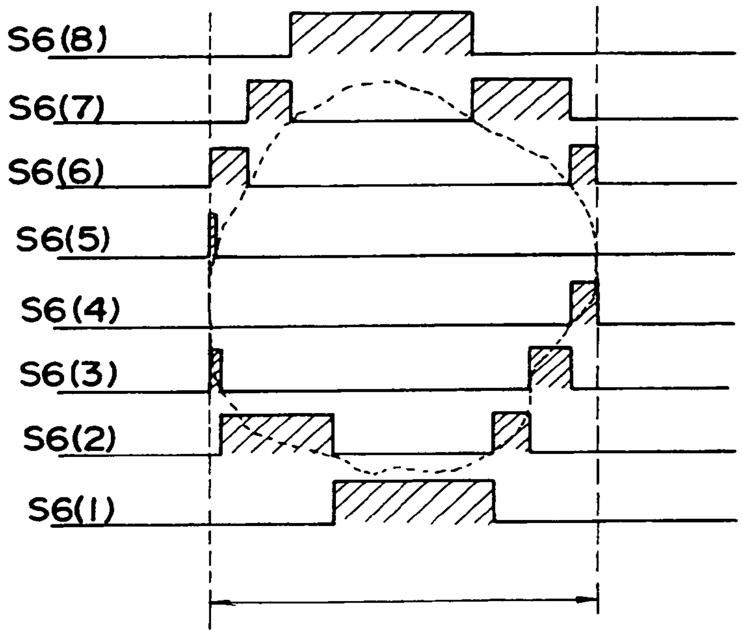
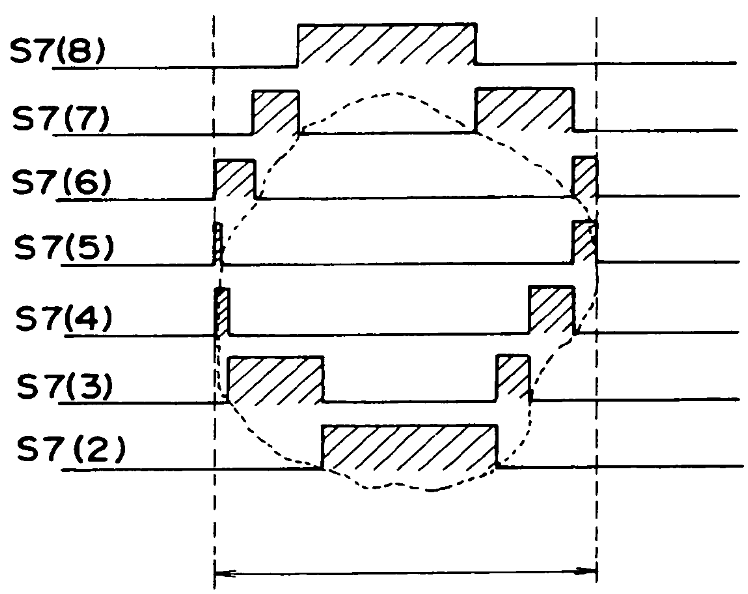


FIG.24



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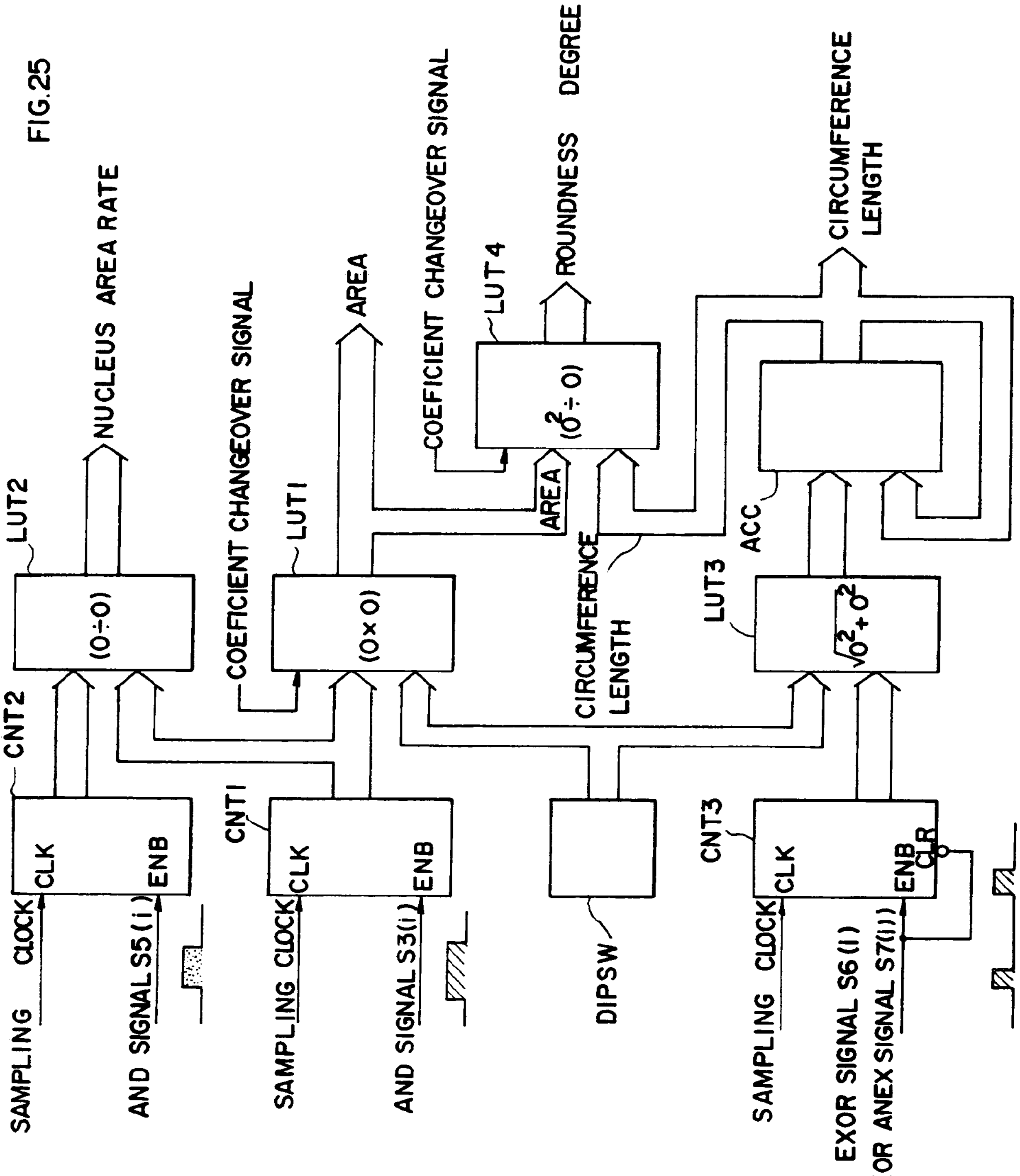

FIG.25

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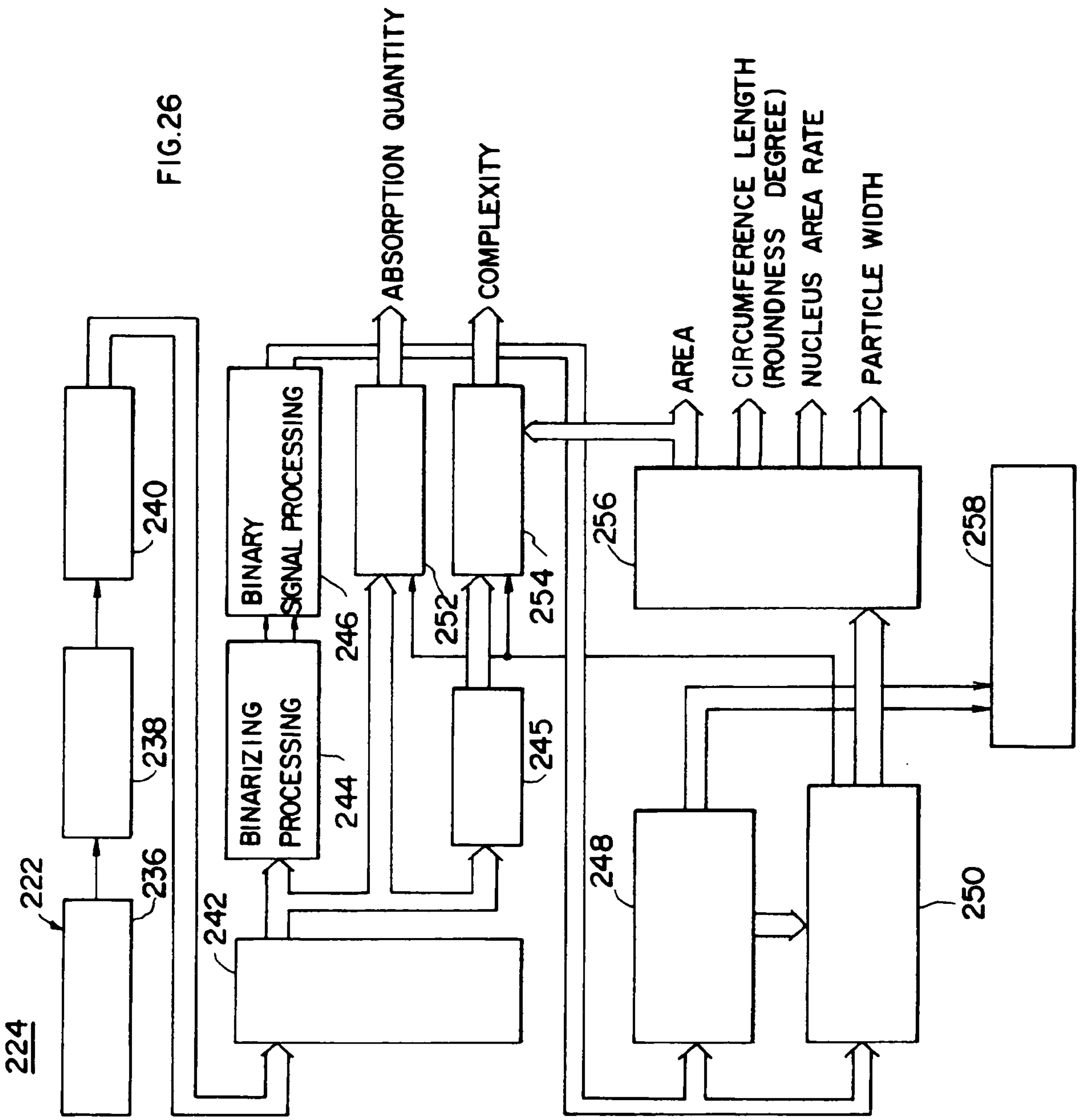
FIG.26



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(54) **Particle analyzer.**

(57) The invention comprises a particle analyzer for discharging a liquid specimen flow containing particles from a nozzle of a flow cell (14), forming a sheath flow by passing a sheath liquid around the liquid specimen flow, directing a first light beam (e.g. laser beam) at the liquid specimen flow, detecting the first light beam after it has interacted with the particles in the liquid specimen flow, and analyzing the particles on the basis of the detected first light beam, wherein the liquid specimen flow is a flat flow which is thin in the direction of the first light beam and wide in the direction transverse to the first light beam, and the particle analyzer comprises: a one-dimensional image sensor (22) which extends transversely of the particle flow direction for having focused thereon particle image(s) produced by the first light beam and is arranged to be scanned to produce for every scan cycle an image signal corresponding to the particle image(s) focused on the image sensor (22); and signal processing means (24, 25) for processing the image signals produced by one-dimensional image sensor (22).

The particle analyzer is excellent at calculating morphological information of particles in a liquid specimen such as blood and urine.

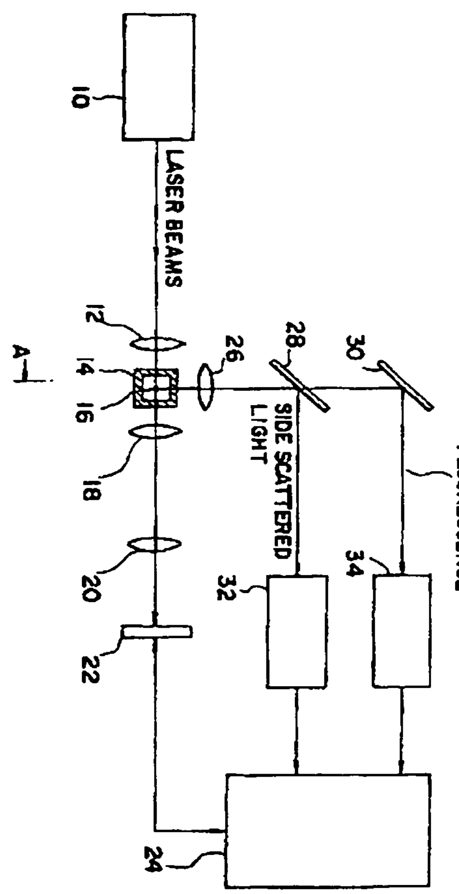

FIG.2

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	US-A-4 643 566 (OHE ET AL.) * column 1, line 14 - line 21; figure 1 * * column 1, line 34 - line 48; figure 3 * * column 3, line 8 - line 49 * ---	1	G01N15/14
A	EP-A-0 443 700 (MITSHUBISHI JUKOGYO K.K.) * page 8, line 30 - line 58; figures 1A-C * ---	1	
A	US-A-4 293 221 (KAY ET AL.) * column 15, line 43 - line 53; figure 9 * ---	1	
A	EP-A-0 361 503 (TOA MEDICAL ELECTRONICS) * column 12, line 19 - column 13, line 55; figure 5 * * column 14, line 53 - column 15, line 5 * ---	7	
A	EP-A-0 158 409 (THE BRITISH LIBRARY BOARD) * page 1, line 13 - line 21; figures 1,2 * * page 2, line 2-7 * * page 5, line 4 - page 6, line 11 * ---	7	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	EP-A-0 361 504 (TOA MEDICAL ELECTRONICS) * page 7, line 35 - page 8, line 12; figures 3,13 * * page 6, line 3-6 * * page 7, line 35 - line 53; figure 3 * * page 7, line 54 - page 8, line 12; figure 13 * ---	7	G01N H04N
A	EP-A-0 039 899 (HITACHI) * page 6, line 13 - page 8, line 2; figure 1 * * page 9, line 6 - page 10, line 3; figure 2 * --- -/--	7	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 AUGUST 1993	ZINNGREBE U.

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- [ ] All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- [ ] Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

  namely claims:
- [ ] No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

- [x] All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- [ ] Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

  namely claims:
- [ ] None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

  namely claims:

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 275 143 (OKI ELECTRIC INDUSTRY COMPANY) * column 3, line 62 - column 5, line 14; figure 8 * * column 8, line 12 - line 17 * -----	7	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 AUGUST 1993	ZINNGREBE U.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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P : intermediate document

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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

EP 92 30 8575 -B-

LACK OF UNITY OF INVENTION A POSTERIORI

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims 1-6:

   Particle analyzer with flat specimen flow, one-dimensional image sensor and second imaging means for capturing two-dimensional still images

2. Claims 7-18:

   Particle analyzer with flat specimen flow, one-dimensional image sensor and specially adapted signal processing means

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